Longitudinal Impedance of Skinned Frog Muscle Fibers

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ABSTRACT Longitudinal impedance of skinned muscle fibers was measured with extracellular electrodes and an oil gap method in which a central longitudinal section of fiber is insulated by oil while the ends of the fiber are bathed in conducting pools of relaxing solution. Intact single fibers were isolated from frog semitendinosus muscle and the sarcolemma removed either by mechanical or chemical methods. Stray capacitance across the oil gap was measured after each experiment and its admittance subtracted from the admittance of the fiber and oil gap. Effects of impedance at the ends of the fiber were eliminated by measuring the impedance with two lengths of fiber in the oil gap and subtracting the impedance at the shorter length from that at the longer length. Longitudinal impedance so determined for mechanically and chemically skinned fibers exhibited zero phase shift from 1 to 10,000 Hz, i.e., the longitudinal impedance of skinned fibers is purely resistive. If we assume that our skinned fibers are a model of the sarcoplasm of muscle, we conclude that the equivalent circuit of the sarcoplasm is a resistor.

INTRODUCTION

The linear passive electrical properties of muscle fibers are important in the function of muscle: they determine the radial spread of potential in the tubular system, which is an important step in excitation-contraction coupling, and they help determine the shape and conduction velocity of the action potential. The linear passive properties are also interesting in themselves since they specify the properties of individual membranes and systems which make up a muscle fiber. Many of the linear properties of muscle have been determined by measuring the impedance of fibers to alternating current applied with a microelectrode (Falk and Fatt, 1964; Freygang et al., 1967; Schneider, 1970; Valdiosera et al., 1974 c). The relation between the electrical model used to describe the linear properties and the structure of the muscle fiber deserves detailed consideration (Valdiosera et al., 1974 b) but for our purposes the model can be considered to be a one-dimensional electrical cable consisting of elements (the longitudinal impedance) which conduct current longitudinally

down the fiber and elements (the shunt impedance) which conduct current out of the fiber into the extracellular solution.

The impedance that is measured in an intact fiber depends both on the longitudinal and shunt impedances, but it is important to know the longitudinal and shunt impedances separately, for several reasons. First, each impedance represents the properties of different structures within the fiber and each has a different effect on the nonlinear active properties of muscle fibers, such as the action potential and excitation-contraction coupling. Second, the impedance of muscle fibers has been extensively studied and interpreted assuming the longitudinal impedance to be purely resistive; but if the longitudinal impedance were not purely resistive, the circuit model, the values of the components of the model, and the anatomical correlates of the components of the model would all be called into question. Finally, there is hope that measurements of the longitudinal impedance of muscle fibers might reveal some of the electrical properties of the sarcoplasmic reticulum, which are of considerable physiological interest.

We have attempted to measure directly the longitudinal impedance of muscle fibers by using apparatus and a preparation which minimizes the effects of current flowing through the shunt impedance. The apparatus was designed (Cole and Hodgkin, 1939) so that current flows longitudinally down a substantial length of the fiber; the effect of the shunt impedance is essentially restricted to the ends of the fiber and can be kept small. The preparation used in the experiments reported here is a muscle fiber with the surface membrane removed, the "skinned" fiber preparation of Natori (1954). In this preparation the shunt impedance should be negligible since there is no surface membrane.

Freygang and Trautwein (1970) have reported the existence of longitudinal reactance in cardiac Purkinje fibers; Mobley et al. (1973) found longitudinal reactance in intact skeletal muscle fibers although Schneider (1970) did not. This work prompted the experiments reported here.

METHODS

Isolation of Single Muscle Fibers

Single fibers of the dorsal head of the semitendinosus muscle of the frog *Rana pipiens* were isolated in Ringer solution (NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; Tris, 3.7; pH 7.2; concentrations in mM), with tendon being left at each end of the fibers. Very large frogs were chosen since they had muscle fibers of diameter up to 160 μm. After their isolation, fibers were left undisturbed at least an hour, though often overnight at 4°C, and they were examined in a dissecting microscope and stimulated electrically at several places along their length. Experiments were performed on fibers which gave propagated twitches from stimulation at each location and which were without visible damage. All experiments were performed at a temperature of 21°C.

Mechanical Skinning

Intact single fibers were placed in a relaxing solution of composition (mM): K^+ methanesulfonate, 120; $MgCl_2$, 1; Na_2ATP, 4; K_2EGTA, 2; imidazole, 10; pH 7.0. A knife was used to make a small transverse cut into the fiber near one of the tendons; cut tissue was grasped with forceps and a sleeve of material was torn from the fiber by moving the forceps towards the other tendon (see Fig. 1, Podolsky, 1968). The material torn from the fiber presumably includes extracellular connective tissue, surface membrane, and outer myofibrils. The skinning is asymmetrical: the greater part of the fiber interior that is torn away from the fiber is torn from the side of the fiber that is grasped by the forceps.

The procedure described above was developed by Endo et al. (1970) from the original method of Natori (1954) in which fibers were skinned in oil. Our electrical measurements require long lengths of skinned fiber and we found these much easier to prepare if the fibers were skinned in relaxing solution instead of oil.

Chemical Skinning

Intact single fibers were bathed in relaxing solution for a few minutes and then were soaked for 45 min in a glycerol solution (composition: glycerol 47% vol/vol; K_2EGTA 2 mM; imidazole, 10 mM; pH 7.0). Fibers were placed for another 45 min in relaxing solution to which 0.5% (wt/vol) of Lubrol WX (a nonionic detergent: Sigma Chemical Co., St. Louis, Mo.) was added. The above procedure is similar to that used by Julian (1971), who found that fibers treated in this manner have effectively no sarcolemma.

Experimental Bath and Mounting Procedure

Impedance was measured in a bath (Fig. 1: see Cole and Hodgkin, 1939; Tamasige, 1950; Freygang and Gunn, 1973) designed so that current flows longitudinally down a substantial length of muscle fiber with little opportunity for transverse flow. Each end of the fiber is held in a pool of relaxing solution and a central region of the fiber in a pool of oil, an oil gap. Current applied to one pool with a Pt/Pt black electrode flows into the fiber, down the length of the fiber in the oil gap, and out the other end of the fiber through the relaxing solution and into another Pt/Pt black electrode. Some current also flows in a stray capacitance between the two pools of relaxing solution.

The apparatus is designed to allow convenient mounting of the fiber and consists of three vessels: a movable test tube enclosing the upper pool of relaxing solution, a movable outer cylinder enclosing the central pool of oil, and a fixed inner cylinder enclosing the lower pool of relaxing solution. A movable rod placed into the test tube allows vertical adjustment of the position of the fiber in the test tube.

The mounting procedure is important to the success of the experiments. A thread is strung from the rod in the test tube through a hole (diameter 645 μm) drilled in the bottom of the test tube. The outer cylinder of the bath is filled with oil (silicone or paraffin) and lowered to allow easy access to the inner cylinder; an "O" ring mounted on the base of the outer cylinder provides a seal and permits vertical movement. The test tube is lowered until it touches the relaxing solution in the inner

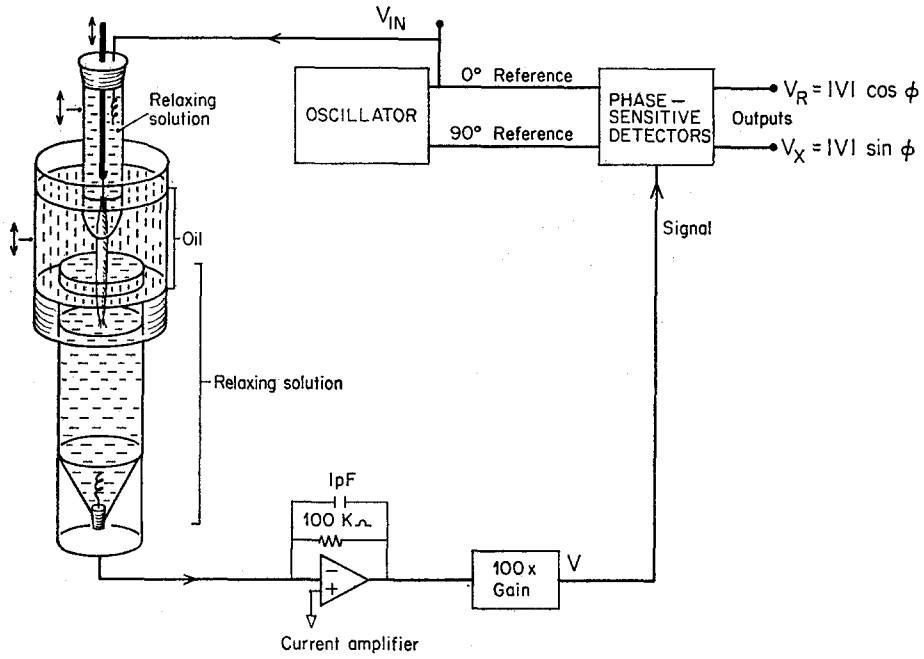

FIGURE 1. A diagram of the experimental bath and electronic apparatus used to determine the longitudinal impedance of skinned fibers. Each end of the fiber was held in a conducting pool of relaxing solution while a central longitudinal section was insulated by the oil gap. V_{IN}, V_R, and V_X were measured on a digital voltmeter (John Fluke Mfg. Co. Inc., Seattle, Wash., model 8200 A) and printed (Systron-Donner Corp. Datapulse Div., Culver City, Calif., model 5103). Independent vertical movement of three pieces: the rod in the test tube, the test tube, and the outer cylinder, is indicated by the double arrows.

cylinder. After skinning, the diameter of the fiber is measured with an eyepiece graticule in a dissecting microscope; then, the tendons of the fiber are held close together and the fiber is quickly transferred through air into the inner cylinder. The thread projecting through the hole in the test tube is tied to a tendon of the fiber and some 3 mm of fiber pulled into the test tube by moving the rod upwards. The outer cylinder is then raised until a substantial part of the test tube is bathed in oil; the relaxing solution in the inner cylinder is thus covered by oil. An oil gap is then formed by raising the test tube, pulling a central part of the fiber into oil. The length of the gap can easily be measured and adjusted. Changes in the length of the gap which are made by moving the test tube, change the length of the fiber in the lower pool of relaxing solution as well.

Electronic Apparatus

Sinusoidal current in the range of frequencies 1–10^5 Hz was applied to one electrode in the bath from a low distortion oscillator (Optimation, Chatsworth, Calif., model RCD-709, special). The other bath electrode was connected to the inverting input of a wide-band, low noise, operational amplifier (Teledyne Philbrick, Dedham, Mass.,

model 1027). Further amplification of voltage was obtained from two cascaded operational amplifiers each connected to have a gain of −10. The output was applied to two phase sensitive detectors (Brookdeal model 411, % Ortec, Inc., Oak Ridge, Tenn.) arranged to measure the real and imaginary parts of impedance as described by Valdiosera et al., 1974 *a*).

Calibration of the System

The phase sensitive detectors and amplifiers introduce phase shift and frequency-dependent gain into any signal measured by the system, so it is necessary to measure and correct for this effect. A calibration curve for the apparatus was obtained in the following manner: at low frequencies (below 22 Hz) the solutions filling the apparatus were made purely resistive (distilled water was used) and the deviations from the properties of a pure resistor were measured. At higher frequencies an oil gap, with relaxing solution in the end pools, was used and the deviation from the properties of a pure capacitor were measured. The deviations are plotted in Fig. 2. An air gap instead of an oil gap gave the same results. The calibration curve did not change measurably during the course of the experiments.

Procedures and Corrections for Capacitive Artifact and End Effects

The impedance $\mathbf{Z}_{obs}(x,\omega)$ of a skinned fiber and apparatus was measured at two gap lengths, x_1 and x_2, in the range of frequencies 1–10^5 Hz at 6 frequencies per decade.

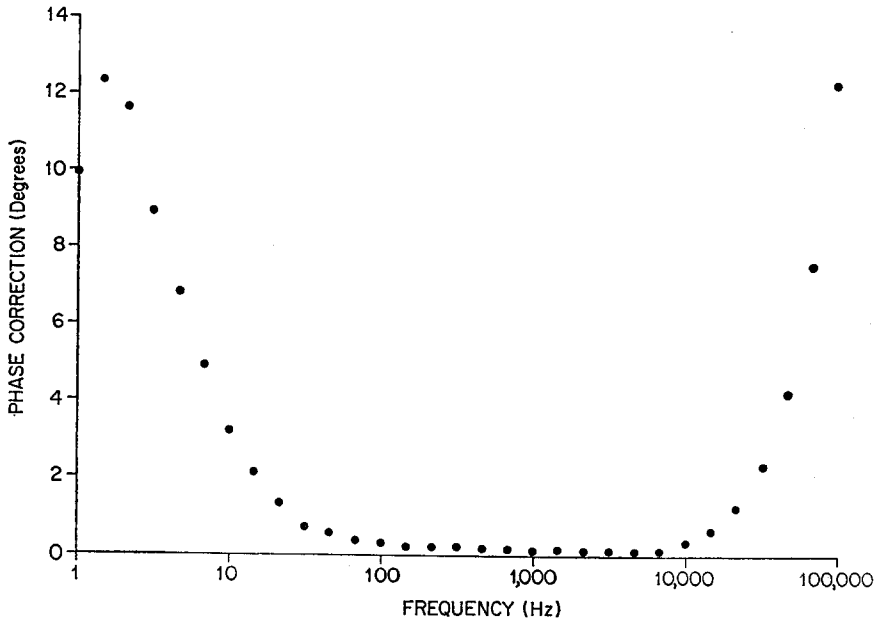

FIGURE 2. Phase calibration of the electronic system. The phase-sensitive detectors are primarily responsible for the deviation at low frequencies and the amplifiers are primarily responsible for the deviation at high frequencies.

$\mathbf{Z}_{obs}(x,\omega)$ (Fig. 3) consists of the impedance of the ends of the fiber, $\mathbf{Z}_1(\omega)$ and $\mathbf{Z}_2(\omega)$, and the impedance of the length of fiber in oil $\mathbf{Z}_f(x,\omega)$, in parallel with the stray capacitance between the pools of relaxing solution indicated by the admittance $\mathbf{Y}_c(x,\omega)$. $\mathbf{Z}_1(\omega)$ and $\mathbf{Z}_2(\omega)$ are assumed to be independent of gap length, but $\mathbf{Z}_f(x,\omega)$ and $\mathbf{Y}_c(x,\omega)$ depend on the length of the oil gap.

The impedance of a length of fiber, $\Delta x = x_2 - x_1$, in the oil gap can be determined by the following procedure: determine $\mathbf{Z}_{obs}(x,\omega)$ at two different gap lengths from the observed voltage and current corrected for the gain and phase shift of the apparatus. Remove the fiber from the bath and measure the capacitive admittance at the two gap lengths x_1 and x_2 at a frequency of 10^4 Hz, then calculate $\mathbf{Y}_c(x,\omega)$ at the other frequencies.

The longitudinal impedance of the length Δx of the fiber in the oil gap is given by:

$$\mathbf{Z}_f(\Delta x,\omega) = \mathbf{Z}(x_2,\omega) - \mathbf{Z}(x_1,\omega), \tag{1}$$

where $\mathbf{Z}(x,\omega)$ is the impedance of the ends and middle of the fiber.

$$\mathbf{Z}(x,\omega) = \frac{1}{\frac{1}{\mathbf{Z}_{obs}(x,\omega)} - \mathbf{Y}_c(x,\omega)} = \mathbf{Z}_f(x,\omega) + \mathbf{Z}_1(\omega) + \mathbf{Z}_2(\omega). \tag{2}$$

Test of Procedure and Methods of Analysis

When the impedance of biological tissue is measured, instability and nonlinearity are of great concern; they are of special concern here because the subtraction procedure used to calculate the final result (see Eq. 1) would magnify their effects. Therefore experiments were performed on two fibers (Fibers nos. 4 and 5 in Table I) to test the assumption of linearity and stability. $\mathbf{Z}_{obs}(x,\omega)$ was measured as usual, and then about 20 min later was remeasured at a signal level one-half that previously used. Plots of the phase angle of the impedance, $\angle \mathbf{Z}_{obs}(x,\omega)$, were similar to each other; the difference at any one frequency was typically 0.1° or 0.2°, and a maximal difference of 3° occurred in one of the experiments at a frequency of 10^5 Hz. If we limit our consideration to frequencies 10^4 Hz, or less, the maximal difference was 0.7° in one fiber at the highest frequency. It is our impression that the stability of these skinned preparations is good, the phase angle of the impedance not changing significantly in several hours. It was not feasible to test the stability of our fibers by measuring impedance sequentially at gap lengths of x_1, x_2, and again at x_1 because there was difficulty in returning to a gap length of exactly x_1.

Another important assumption is the applicability of the equations used to analyze the results (Eqs. 1 and 2). These equations assume that the end effects $\mathbf{Z}_1(\omega)$ and $\mathbf{Z}_2(\omega)$ are independent of gap length; equivalently they assume that $\mathbf{Z}(x,\omega)$ is a linear function of gap length. Fig. 4 shows a plot of the real part of $\mathbf{Z}(x,\omega)$ (called the effective resistance, R) as a function of gap length (nine lengths were used). Points along the dashed line (○) show the resistance, R, obtained at each gap length; each point is the mean R measured at the frequencies 1, 10, 10^2, 10^3, and 10^4 Hz and the indicated gap lengths; each symbol (●) on the continuous line represents R measured at 10^5 Hz and the indicated gap lengths. The imaginary part of $\mathbf{Z}(x,\omega)$ (called the

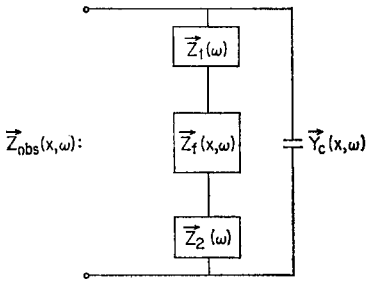

FIGURE 3

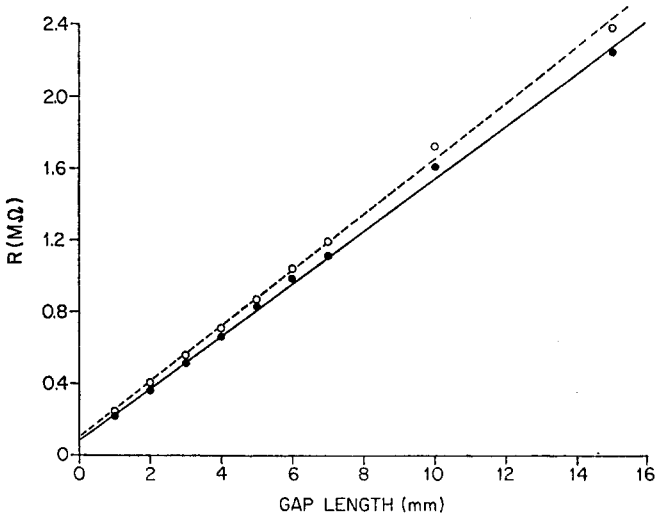

FIGURE 4

FIGURE 3. A diagram indicating the impedances composing $\mathbf{Z}_{obs}(x,\omega)$, the impedance measured directly. The elements of the circuit are described in the text.

FIGURE 4. A plot of R, the real part of $\mathbf{Z}(x,\omega)$, as a function of gap length for one of our fibers; the experiment shows that $\mathbf{Z}(x,\omega)$ is a linear function of gap length, and it justifies the subtraction procedure of Eqs. 1 and 2 used to analyze the results. Each point along the dashed line (○) shows the mean R measured at the frequencies 1, 10, 10^2, 10^3, and 10^4 Hz and each point along the continuous line (●) shows R measured at 10^5 Hz. The fiber is no. 10 in Table I. The lines are regression lines calculated by the method of least squares; correlation coefficients are 0.999 for both lines.

effective reactance, X) was also plotted as a function of gap length for this fiber; the plot showed that the reactance is a linear function of gap length; the slopes and intercepts with the ordinate were very small at the frequencies 1, 10, 10^2, 10^3, and 10^4 Hz. The maximum slope = 0.002 MΩ/mm (10^4 Hz) and the maximum intercept = −0.0142 MΩ (1 Hz). At 10^5 Hz the slope of the reactance versus gap length is approximately 0.015 MΩ/mm and the intercept is −0.027 MΩ at 10^5 Hz. The intercept with the ordinates, X or R, (Fig. 4), is only a crude estimate of the constant end effects, $\mathbf{Z}_1(\omega) + \mathbf{Z}_2(\omega)$; there are significant errors in our measurement of a gap length of zero. We conclude that the preparation behaves as a combination of stable and linear circuit elements as described by Eqs. 1 and 2.

Physiological State of the Preparation

In order to compare the state of our skinned fibers with those of other investigators we tested the mechanical response of the preparation to variations in the concentration of calcium ions and to the application of caffeine. The tension of mechanically and chemically skinned fibers could be changed by varying the concentration of calcium ions; the threshold for development of tension was approximately 10^{-7} M and a maximal tension was developed at a calcium concentration of approximately 10^{-6} M (Hellam and Podolsky, 1969; Julian, 1971). We observed maximal tensions of 0.65

kg/cm² * for mechanically skinned fibers and 1.0 kg/cm² * for chemically skinned fibers. Transient caffeine contractures were obtained on mechanically skinned fibers (Endo et al., 1970), but not on chemically skinned fibers, the few times we tried to elicit them.

RESULTS

Mechanically Skinned Fibers

The data of most direct physiological interest is the longitudinal impedance of a length of fiber entirely within the oil gap, $\mathbf{Z}_f(\Delta x,\omega)$. Since this quantity is derived from the experimentally observed impedance, $\mathbf{Z}_{obs}(x,\omega)$, using corrections for the capacitive admittance and the end effects (Eqs. 1 and 2), we show the complete data for one fiber (Fig. 5). Fig. 5 *a* is a plot of the phase angle of $\mathbf{Z}_{obs}(x,\omega)$ (indicated by the symbol $\angle\mathbf{Z}_{obs}(x,\omega)$) at a gap length of 7 mm; Fig. 5 *a* also shows the phase angle of the impedance of the preparation after the correction for capacitive artifact is made (Eq. 2), $\angle\mathbf{Z}(x,\omega)$. Fig. 5 *b* shows similar plots for the same fiber at a gap length of 14 mm, and Fig. 5 *c* is a plot of $\angle\mathbf{Z}_f(\Delta x,\omega)$ derived by taking the difference between $\mathbf{Z}(x_2,\omega)$ and $\mathbf{Z}(x_1,\omega)$ (Eq. 1). These three plots are typical of our results.

Capacitive, that is, negative phase angles were observed in $\mathbf{Z}_{obs}(x,\omega)$ in the range of frequencies 10^3–10^5 Hz in all experiments. The phase shift between 10^3 and 10^4 was caused by the capacitive artifact: when $\mathbf{Z}_{obs}(x,\omega)$ was corrected for the capacitive artifact the resulting impedances $\mathbf{Z}(x,\omega)$ and $\mathbf{Z}_f(\Delta x,\omega)$ had essentially zero phase. The negative phase angle observed above 10^4 Hz was only partly the result of the capacitive artifact: when the observed impedance was corrected for the artifact, positive ("inductive") phase angles were found in $\mathbf{Z}(x,\omega)$ and $\mathbf{Z}_f(\Delta x,\omega)$. We are unsure of the significance of this result because the possibility of unknown errors in this range of frequencies is considerable. Since phenomena of direct physiological interest do not occur in this range of frequencies and since equipment was not available to work at higher frequencies, as would be needed to properly investigate the positive phase angle, most of our data and conclusions are drawn from results at frequencies below 10^4 Hz. We do, however, investigate in the next section the possibility that the positive phase shift is associated with current flow across internal membranous structures.

Data on $\mathbf{Z}_f(\Delta x,\omega)$ for 10 mechanically skinned fibers is presented in Table I. $\langle|\mathbf{Z}_f(\Delta x,\omega)|\rangle$ in each row is the mean of the magnitude of $\mathbf{Z}_f(\Delta x,\omega)$ measured at 25 frequencies between 1 and 10^4 Hz; $\langle\angle\mathbf{Z}_f(\Delta x,\omega)\rangle$ is similarly a mean of all phase angles observed for each fiber. The largest value of the phase angle (neglecting sign) for each fiber in the range of frequencies 5–10^4 Hz is also given. The phase angle at lower frequencies is generally larger than that

* Fibers may swell after they are skinned.

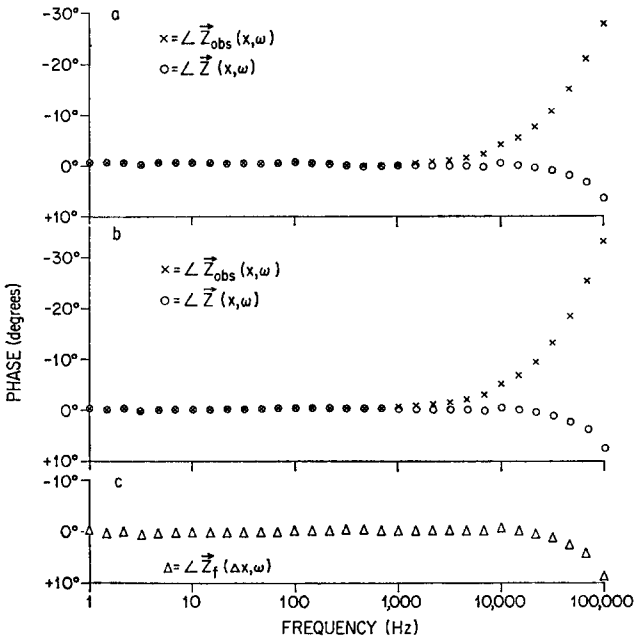

FIGURE 5. Complete phase data for one of our mechanically skinned fibers (Fiber no. 6 in Table I). (*a*) Phase angle of $\mathbf{Z}_{obs}(x,\omega)$ (×) and $\mathbf{Z}(x,\omega)$ (○) with respect to frequency, gap length = 7 mm, I_{rms} = 73 × 10^{-9} A, C_{gap} = 1.24 pF. (*b*) Phase angle of $\mathbf{Z}_{obs}$ (x,ω) (×) and $\mathbf{Z}(x,\omega)$ (○) with respect to frequency, gap length = 14 mm, I_{rms} = 72 × 10^{-9} A, C_{gap} = 0.81 pF: (*c*) Phase angle of $\mathbf{Z}_f(\Delta x,\omega)$ with respect to frequency. Nine other fibers gave similar results (Table I).

shown, but experimental errors associated with the long integration times required by the phase-sensitive detectors are most likely responsible. The internal resistivity, R_i, was calculated at each of the 25 frequencies for each fiber assuming the fiber was circular in cross section and the mean of those values is given. The average value of R_i from all 10 fibers, 167 ohm-cm, is surprisingly close to the value 169 ohm-cm determined by Hodgkin and Nakajima (1972) from single intact muscle fibers considering that fibers may swell after skinning and that the cross-sectional size and shape of the fiber were not measured while the fiber was in the oil gap. R_i for other types of skinned fibers are presented in the appropriate figure legends.

We were concerned that a layer of relaxing solution trapped between the oil and the myofibrils would be a resistive shunt to the longitudinal impedance of fiber in the gap and would affect our measurement of impedance. We could not measure the thickness of the layer, but because the R_i we measured is close to R_i of intact fibers, it is unlikely that the layer significantly affects our measurements.

We conclude that the phase angle of the longitudinal impedance is indis-

TABLE I

$Z_f(\Delta x,\omega)$ FOR MECHANICALLY SKINNED FIBERS

Fiber no.	x_1	x_2	Diameter	$\langle \lvert Z_f(\Delta x,\omega) \rvert \rangle$	$\langle \angle Z_f(\Delta x,\omega) \rangle$	$\langle R_i \rangle$	Maximum phase
	mm	*mm*	*μm*	*MΩ*	*degrees*	*ohm-cm*	*degrees*
1	6.0	11.0	127	0.544	−0.10	138	0.32
				(0.003)	(0.51)	(1)	
2	4.0	14.0	80	3.749	0.14	188	0.45
				(0.125)	(0.28)	(6)	
3	4.0	9.0	73	1.519	0.11	127	0.31
				(0.031)	(0.27)	(3)	
4	8.0	16.0	109	1.122	0.17	131	0.39
				(0.151)	(0.36)	(18)	
5	6.0	13.0	127	1.312	0.11	238	0.35
				(0.025)	(0.27)	(4)	
6	7.0	14.0	146	0.761	0.08	182	0.37
				(0.012)	(0.29)	(3)	
7	5.0	11.0	146	0.707	0.11	197	0.69
				(0.004)	(0.30)	(1)	
8	6.0	11.0	109	0.567	0.18	106	0.57
				(0.019)	(0.46)	(4)	
9	5.0	9.0	91	0.895	0.08	145	0.38
				(0.018)	(0.32)	(3)	
10	10.0	15.0	146	0.659	0.23	221	1.15
				(0.006)	(0.66)	(2)	
		Mean	115			Mean 167 (14; n = 10)	

Numbers in parentheses are standard deviations.

⟨ ⟩ denote the mean value of the enclosed parameter.

The maximum phase is the largest value of $\angle Z_f(\Delta x,\omega)$, ignoring sign, in the frequency range 5–10^4 Hz.

x_1, x_2 are the gap lengths.

tinguishable from zero at frequencies between 1 and 10^4 Hz and therefore that the longitudinal impedance of the skinned fibers is purely resistive. The resistivity of skinned fibers is close to the internal resistivity of intact fibers.

Mechanically Skinned Fibers Treated with Detergent

Impedance was measured on three fibers which had been mechanically skinned and then bathed in relaxing solution containing 0.5% (wt/vol) Lubrol WX (See Methods) for 20–45 min. Skinned fibers treated with detergent are difficult to work with since they are fragile; they often break under their own weight in relaxing solution or in the oil gap. We postulated that internal membranes would be destroyed by the detergent (Julian, 1971) and so the phase shift observed above 10^4 Hz might be changed. Fig. 6 is a plot of the phase angle $\angle Z_f(\Delta x,\omega)$ for one of the fibers. The results from all three fibers are similar to those reported in Fig. 5 and Table I: the phase angle is zero below

10^4 Hz and positive above. We conclude therefore, that it is unlikely that the phase shift at higher frequencies is produced by current flow through internal membranous structures.

Chemically Skinned Fibers

Fig. 7 shows a plot of the phase angle of the longitudinal impedance $\mathbf{Z}_f(\Delta x, \omega)$ and demonstrates the resistive nature of the impedance in chemically skinned fibers. Similar results were obtained from two other fibers. Chemically skinned fibers are not as stable as fibers that are mechanically skinned; indeed one of

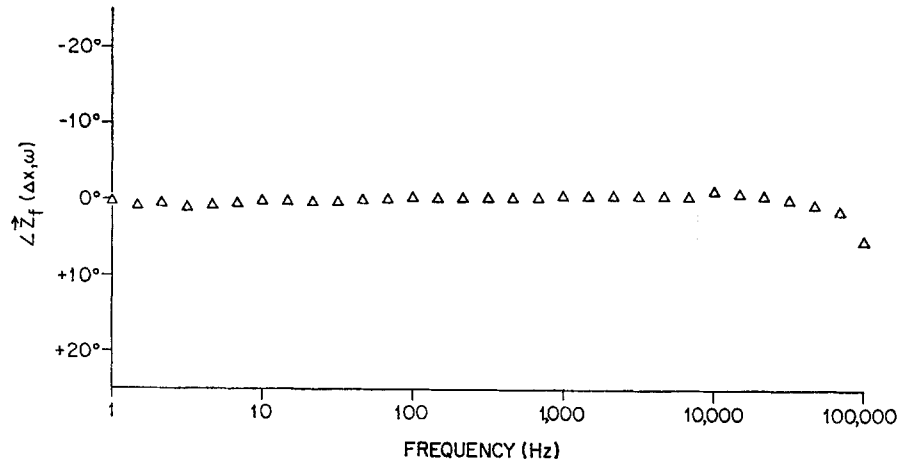

FIGURE 6. Phase angle of the longitudinal impedance, $\mathbf{Z}_f(\Delta x,\omega)$, with respect to frequency. Before it was tested, the fiber was mechanically skinned and then bathed in relaxing solution containing detergent. The two gap lengths were 5.0 and 10.0 mm; R_i = 159 ohm-cm. Two other fibers gave similar phase plots and R_i was 171 and 129 ohm-cm.

the fibers studied was obviously contracting slowly during the experiment. The impedance of this fiber was, nonetheless, essentially identical to that shown in Fig. 7. Chemically skinned fibers were not as fragile as the fibers that were mechanically skinned and subsequently treated with detergent.

Fibers Skinned in Hypertonic Relaxing Solution

Single intact fibers were placed in relaxing solution to which 1,084 mM sucrose was added; these severely shrunken fibers were then mechanically skinned and they remained shrunken even after skinning. We thought that the sarcoplasmic reticulum might be swollen in such preparations (Birks and Davey, 1969) while the parallel current path through the sarcoplasm would be shrunken; the sarcoplasmic reticulum might then be expected to carry a significant fraction of the longitudinal current. These fibers, however, did not

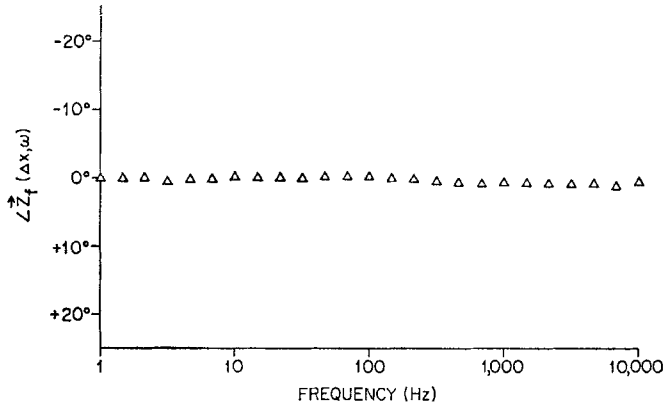

FIGURE 7. Phase angle of the longitudinal impedance, $\mathbf{Z}_f(\Delta x,\omega)$, with respect to frequency. The fiber was chemically skinned. The two gap lengths were 5.0 and 10.0 mm; R_i = 117 ohm-cm. Two other fibers gave similar phase plots and R_i was 124 and 53 ohm-cm.

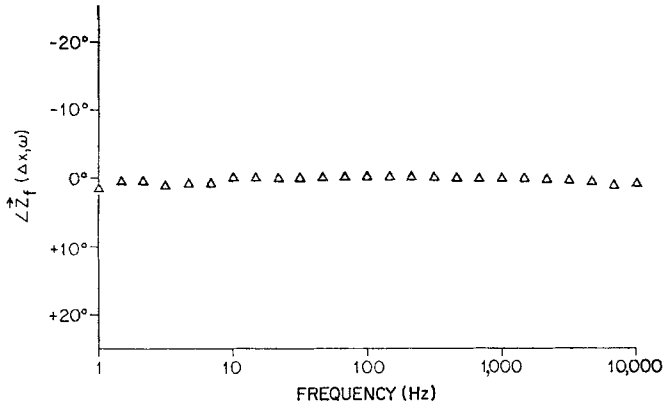

FIGURE 8. Phase angle of the longitudinal impedance, $\mathbf{Z}_f(\Delta x,\omega)$, with respect to frequency. The fiber was mechanically skinned in hypertonic relaxing solution. The two gap lengths were 5.0 and 11.0 mm; R_i = 254 ohm-cm. Another fiber gave a similar phase plot and R_i was 144 ohm-cm.

show longitudinal phase shift (Fig. 8) and so it is unlikely that significant current flowed in the internal membranous structures of the preparation.

DISCUSSION

The longitudinal impedance of skinned muscle fibers is purely resistive, and therefore it is most likely that the impedance of the sarcoplasm of intact muscle fibers is also purely resistive. It is possible, however, that the longitudinal impedance of intact fibers is not purely resistive; significant current might flow longitudinally in the sarcoplasmic reticulum of intact fibers even though significant current apparently does not flow longitudinally in the sarcoplasmic reticulum of skinned fibers.

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Note Added in Proof We recently determined the longitudinal impedance of two fibers mechanically skinned in a relaxing solution containing only 0.1 mM K_2 EGTA. The longitudinal phase angle for these two fibers was zero from 1 to 10^4 Hz (R_i = 138 and 125 ohm-cm). We conclude that the 2 mM EGTA normally used in our experiments did not cause the longitudinal phase angles to be zero.

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